

Supplementary Information

Cerebellar Purkinje cell activity drives motor learning

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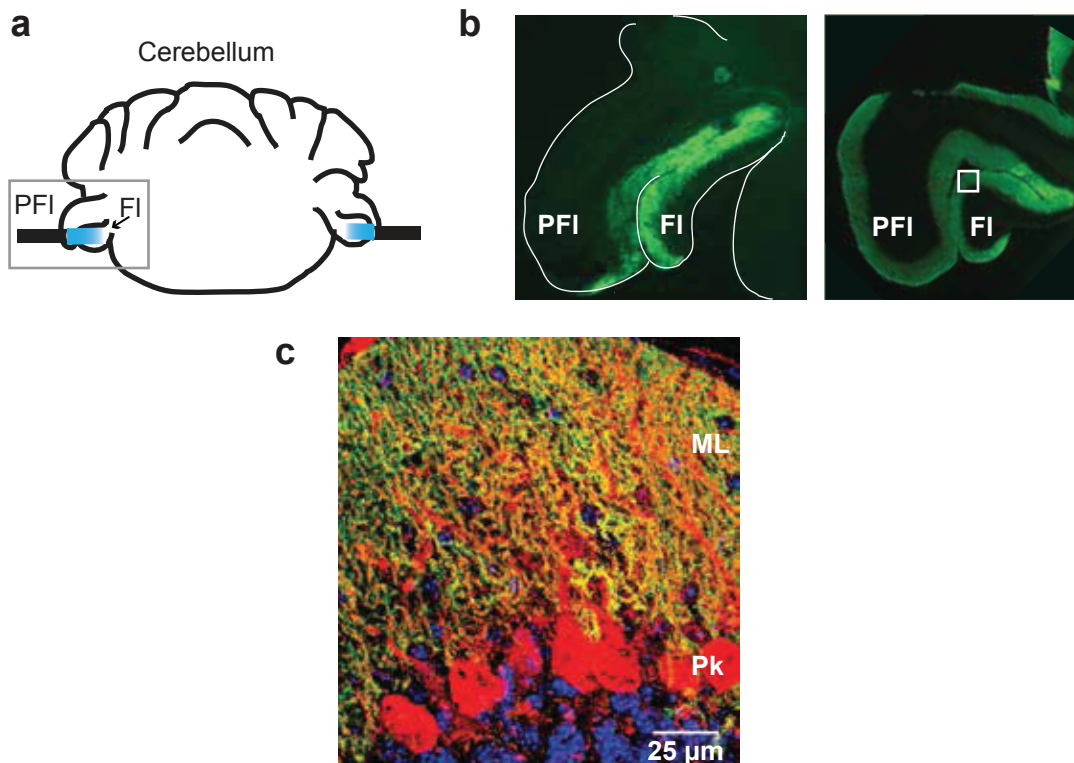
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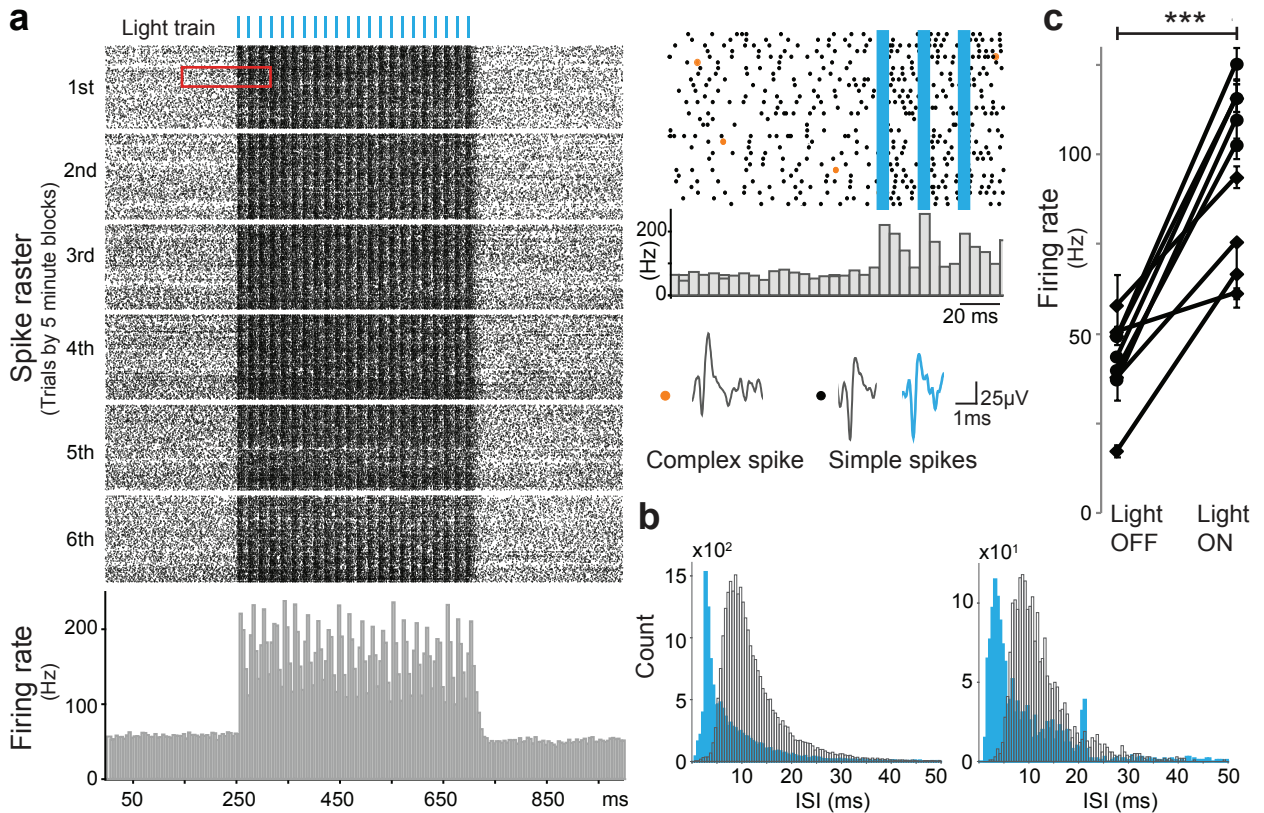
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Supplementary Figures 1-7



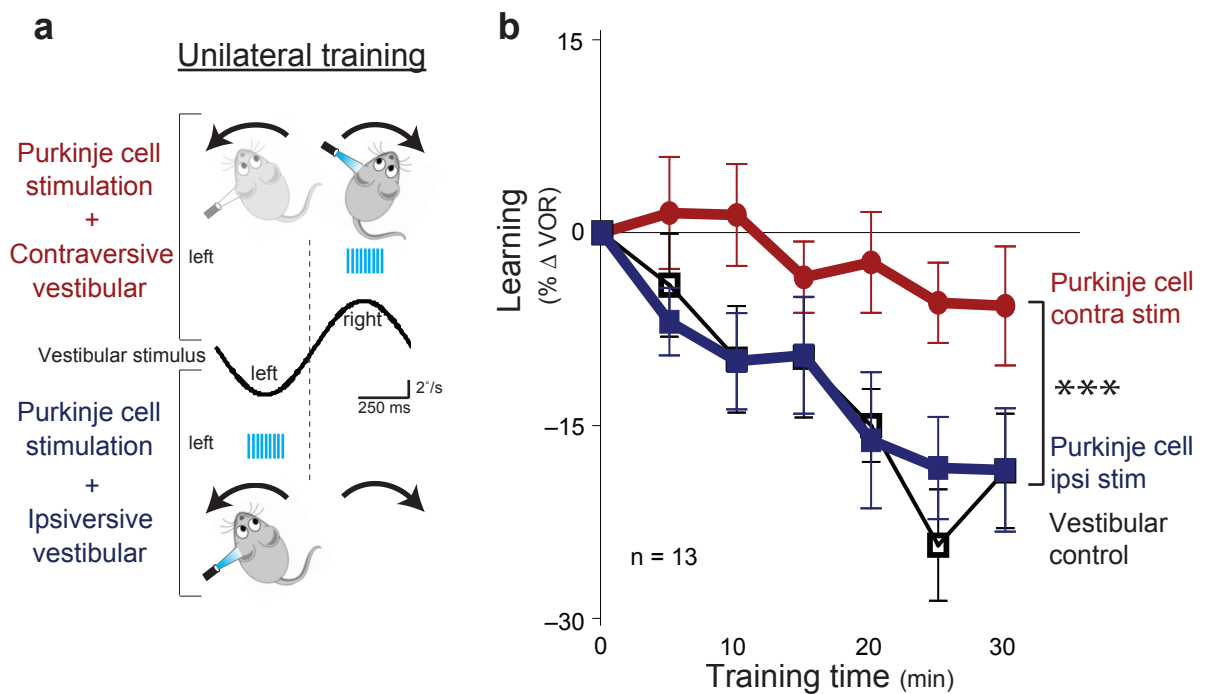
Supplementary Figure 1. Selective expression of ChR2 in Purkinje cells in the cerebellar flocculus

To selectively activate Purkinje cells, we expressed channelrhodopsin (ChR2) under the control of the Purkinje cell-specific L7/*pcp-2* promoter. **(a)** Coronal diagram of the mouse cerebellum showing the site of optogenetic stimulation. An optical fiber delivering 473nm (*blue*) light was sealed to prevent light emission except for where the tip penetrated the brain. FI, flocculus; PFI, paraflocculus. **(b)** Targeting of ChR2-EYFP expression to Purkinje cells of the cerebellar flocculus using viral and transgenic approaches. ChR2-EYFP (*green*) expression in coronal sections corresponding to grey box in (panel a) of an L7-Cre mouse injected with pAAV-EF1a-double floxed-hChR2(H134R)-EYFP (*left*), and a transgenic mouse resulting from a cross between L7-Cre and Ai32 ChR2 mouse lines (*right*). With both approaches, a substantial fraction of Purkinje cells in the flocculus expressed ChR2-EYFP. **(c)** Confocal image of the flocculus of a transgenic mouse (box in panel b, *right*) shows the extensive overlap (*orange*) between the membrane bound protein, ChR2-EYFP (*green*) and antibodies to calbindin (*red*), which labels the Purkinje cells. Blue, cell bodies stained with DAPI; ML, molecular layer; Pk, Purkinje cell layer.



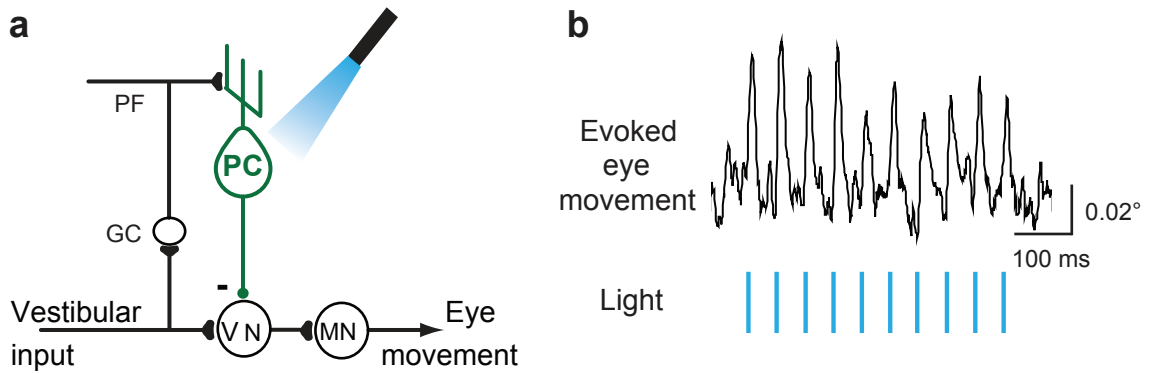
Supplementary Figure 2. Optogenetic activation of Purkinje cells in the cerebellar flocculus

(a) Optrode recordings in awake mice verified that illumination with blue light successfully elicited spiking in Purkinje cells of the flocculus, the region of the cerebellum necessary for VOR learning. In vivo, extracellular optrode recording from an awake animal showing Purkinje cell activation with trains of blue light (trains of 5 ms pulses at 50 Hz for 420 ms every 1 second; 3 mW/mm² intensity). Rasters show the spikes elicited by all the stimulus trains delivered during six 5-min blocks of stimulation (40 seconds between blocks); histogram shows the effect of stimulation over the 30-min (below). Right, expanded view of red box in a, and example spontaneous (black) and ChR2-elicited (cyan) Purkinje cell simple spike waveforms (black dot), which differ from the spontaneous complex spike waveform (orange dot) recorded from the same cell. ChR2-mediated depolarization of Purkinje cells elicited action potentials resembling spontaneous simple spikes, rather than the complex spikes elicited by climbing fiber input, in that they occurred at high rates and did not have the Ca²⁺ spikelets characteristic of spontaneous or optogenetically elicited complex spikes (Compare with Supplementary Figure 6). (b) Histograms of the simple spike inter-spike intervals (ISIs) of two, representative Purkinje cells immediately before the start of stimulation (open bars), and during optogenetic stimulation (cyan bars; trains of 5 ms pulses at 50 Hz for 420ms every 1 second; ≤ 3 mW/mm² intensity). Optogenetic stimulation increased the mean firing rate of the Purkinje cells, as shown by the leftward shift in the ISI distribution, with no evidence of bursting. In some cells, optogenetic stimulation induced some entrainment of firing at the frequency of stimulation (50Hz/20 ms ISI) (right). Data in the left panel are from the same Purkinje cell as in panel a. Bin size, 500 μ s. (c) The increase in Purkinje cell firing rate achieved with optogenetic stimulation was comparable to what has been reported during visually and vestibularly driven eye movements. Blue light stimulation elevated Purkinje cell firing rates (Light ON; 100.1 ± 7.8 Hz) above the spontaneous levels recorded in between the stimulus trains (Light OFF; 45.9 ± 2.6 Hz) ($t(8) = 7.322$, $P < 0.0001$, Paired t-test, $n = 9$ cells from 8 mice; circles, transgenic; diamonds, virus injected). Mean \pm s.e.m.



Supplementary Figure 3. Unilateral Purkinje cell activation induced motor learning

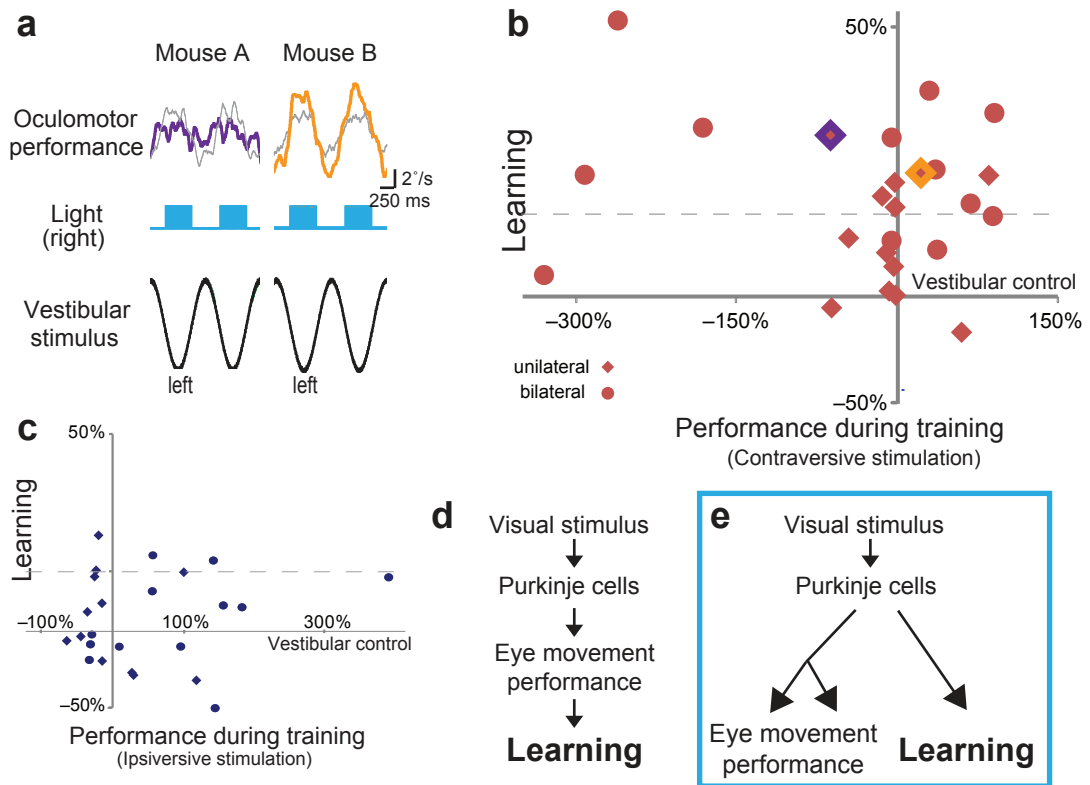
In a separate cohort of animals to that shown in Figure 1, training was done with stimulation of floccular Purkinje cells in only one hemisphere during the contraversive phase of the vestibular stimulus. **(a)** Schematic of the unilateral optogenetic training paradigms for modifying the gain of the VOR. A sinusoidal vestibular stimulus was used for training and testing (black trace, angular velocity of the head). Unilateral optogenetic stimulation of Purkinje cells was paired with the contraversive phase of the vestibular stimulus (*red*, light on when the head was rotating away from the side of Purkinje cell stimulation) or the ipsiversive phase of the vestibular stimulus (*blue*, light on when the head was rotating toward the side of stimulation). **(b)** Motor learning induced by Purkinje cell activation depended on the training condition ($F(2, 36) = 3.513$, $P = 0.04$, two-way repeated measures ANOVA). Motor learning was induced when Purkinje cell activation was paired with contraversive vestibular stimulation (red circles) compared to the vestibular-alone control (*black squares*; $*P = 0.025$, Fisher test; $n = 13$ mice). The learning induced by the unilateral training paradigm was approximately half that induced by bilateral training (compare with Figure 1b, *red circles*). There was no significant difference between training with Purkinje cell stimulation during the ipsiversive phase of the vestibular stimulus (*blue squares*) and the vestibular-alone control (*open squares*; $P = 0.94$, Fisher test; $n = 13$ mice). Instead, the effects on the behavior were associative, since the timing of the Purkinje cell activity relative to the vestibular stimulus was critical to the induction of VOR learning (Purkinje cell contra vs. ipsi stim, $*P = 0.030$, Fisher test; $n = 13$ mice). This is consistent with the previous finding that electrical stimulation of the flocculus induced no learning when paired with stimulation of the ipsilateral vestibular nerve to mimic an ipsiversive vestibular stimulus. Thus, neither the Purkinje cell activation itself, nor any calcium influx or non-associative plasticity it may have caused were sufficient to alter the behavior. None of our optogenetic stimulation paradigms (see Fig. 1 and 2) induced an associative decrease in the VOR below what was induced by the vestibular stimulus alone. Yet, when the vestibular stimulus was paired with an appropriate visual stimulus, a decrease in VOR amplitude below the vestibular-alone control was induced (Supplementary Fig. 7). It is possible that the mechanisms supporting this decrease in the VOR are partially shared with, or otherwise occluded by, the habituation observed in response to the vestibular stimulus alone. On the other hand, a number of recent results suggest that learned increases and decreases in the VOR depend on different mechanisms, so there could be another neural instructive signal, in addition to the Purkinje cells and the climbing fibers, that controls associative VOR decrease learning. Mean \pm s.e.m.



Supplementary Figure 4. Optogenetic stimulation of Purkinje cells elicits eye movements

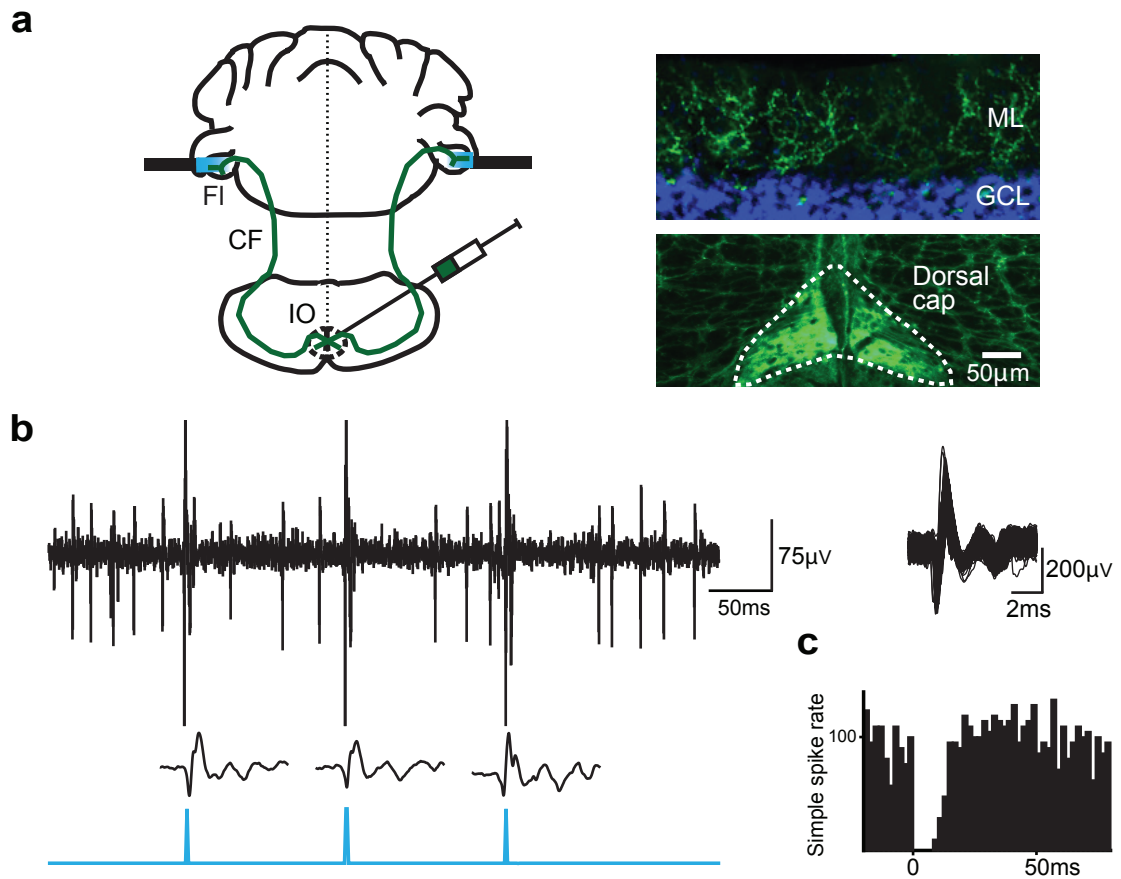
(a) Schematic of the VOR circuit. The inhibitory Purkinje cells (PC) are only two synapses from the oculomotor neurons (MN). GC, granule cell; PF, parallel fiber; VN, vestibular nucleus.

(b) Optogenetic Purkinje cell stimulation elicited eye movement responses at short latencies. When brief (5 ms) light pulses were delivered at a relatively low frequency (20 Hz), the eye movement elicited by each pulse could be resolved, illustrating the effectiveness of Purkinje cell activity to affect online motor performance.



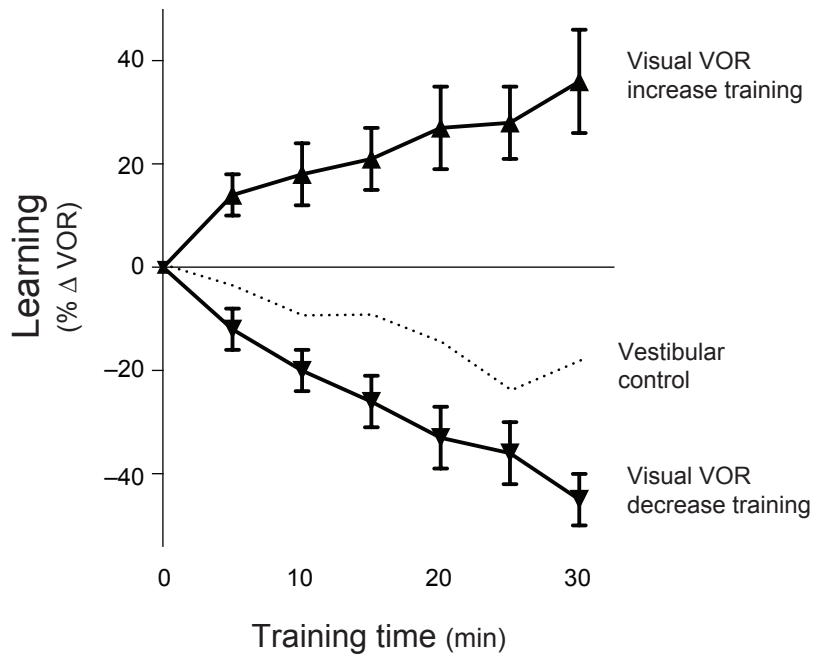
Supplementary Figure 5. Distinct readouts of Purkinje cell activity for motor performance and motor learning

The effects of Purkinje cell activation on the induction of motor learning could be dissociated from its immediate effects on motor performance. **(a)** Examples illustrating the variation across mice in the effects of Purkinje cell stimulation on eye movement performance during training. In different mice, Purkinje cell stimulation during the contraversive phase of the vestibular stimulus could produce an immediate decrease (*left, purple*) or increase (*right, orange*) in eye movement amplitude, compared with the VOR response in the absence of Purkinje cell stimulation (*grey*). In both animals, 30-min of training with Purkinje cell activation during the contraversive phase of the vestibular stimulus induced a learned increase in the VOR, as measured in the absence of Purkinje cell stimulation (*color symbols in panel b*). **(b)** Within a given experiment, there was no significant correlation between the immediate effect of Purkinje cell stimulation on the eye movement performance during training and the learning it induced ($R_{(25)} = -0.17$, $P = 0.42$, Pearson correlation; $n = 25$ mice). Each point represents data from an individual experiment in a different mouse. Learning was measured as the percent change in VOR amplitude at the end of training compared to pre-training, tested in the absence of Purkinje cell stimulation. The effect on performance was calculated as the percent change in eye movement amplitude observed immediately, when Purkinje cells were stimulated during the vestibular stimulus, as compared with responses to the vestibular stimulus alone before training. The immediately evoked eye movements could increase ($n = 9$, *right*), decrease ($n = 10$, *left*), or have no significant effect ($n = 6$, *points lying on vertical axis*, including 4 mice with evoked vertical eye movements) on the amplitude of the ongoing VOR during training. This variable immediate effect on VOR performance mirrors the heterogeneity of the Purkinje cell population within the flocculus, observed in single unit recordings. Variable expression levels of ChR2 and/or the placement of the optical fiber within the flocculus could differentially activate subpopulations of Purkinje cells driving ipsiversive or contraversive eye movements to yield an immediate increase or decrease, and no change if the two populations were activated equally. The immediate effect of Purkinje cell stimulation on eye movement performance was similar at the end versus the beginning of training (1st 5-minute block versus 6th 5-minute block, $t_{(24)} = 1.978$, $P = 0.06$, paired t-test, $n = 2$; data not shown), suggesting that the effectiveness of optogenetic stimulation and Purkinje cell excitability were similar throughout training. Despite variable immediate effects on the ongoing eye movement performance, there was a remarkably consistent effect of Purkinje cell activation on VOR-increase learning (*points above solid horizontal axis, which shows mean for vestibular-alone control*). **(c)** When Purkinje cell stimulation was paired with an ipsiversive vestibular stimulus, there was no significant effect on learning, and there was no significant correlation between the effect of Purkinje cell stimulation on the immediate eye movement performance and learning ($R_{(24)} = -0.26$, $P = 0.22$, Pearson correlation; $n = 25$ mice). **(d)** The results in panels b and c indicate that the effects of Purkinje cell activity on learning is not a secondary consequence of the eye movements present during training, which is evidence against this model. **(e)** Our data support the model that there are independent read outs of the Purkinje cell activity for the control of movement on different time scales: immediate performance versus learning. Different subpopulations of Purkinje cells may make different contributions to eye movement performance, but their contribution to learning appears to be more uniform.



Supplementary Figure 6. Optogenetic activation of climbing fibers in the cerebellar flocculus

The dependence of learning on which cells expressed the ChR2, the Purkinje cells vs. climbing fibers (compare Figures 1 and 2) rules out any non-specific effects of the experimental procedures, such as the presence of the blue light or general activation of the circuit. **(a)** Coronal diagram of the cerebellum and brainstem illustrating the site of virus injection and subsequent optogenetic activation of climbing fibers (green) with 473nm light (left). ChR2 was expressed in climbing fibers by injecting AAV-CaMKII α -hChR2(H134R)-EYFP into the dorsal cap of Kooy, the subnucleus of the inferior olive that provides the climbing fiber input to the flocculus. Right, coronal sections showing ChR2-EYFP expression, visualized using immunostaining against EYFP (green), in the climbing fibers of the flocculus (top) and the corresponding injection site in the dorsal cap of Kooy (bottom). FI, flocculus; CF, climbing fiber; IO, inferior olive; ML, molecular layer; GCL, granule cell layer; Blue, cell bodies stained with DAPI. **(b)** Representative example of an in vivo extracellular optrode recording from a Purkinje cell in the flocculus showing the complex spikes elicited by optogenetic activation of its climbing fiber input (left). Climbing fiber activation was elicited using 250 ms trains of three pulses of blue light (cyan; 2 ms duration, 0.3 mW/mm²) repeated at 1 s intervals, and delivered unilaterally to the cerebellar flocculus. Individual waveforms show the optogenetically elicited complex spikes with the stimulus artifact subtracted. Right, overlay of all the optogenetically elicited complex spike waveforms recorded in this cell during a ~10 minute period of stimulation (1,718 waveforms). Climbing fiber activity has been hypothesized to recruit plasticity in the cerebellar cortex or induce plasticity downstream by causing pauses in Purkinje cell simple spike firing that disinhibit their targets. **(c)** Histogram from a representative Purkinje cell, showing simple spike rate aligned on the time of complex spikes (t=0) elicited optogenetically using 2 ms pulses of light. A typical, 10–20 ms pause in simple spike firing occurred after each optogenetically elicited complex spike. Bin size, 2 ms.



Supplementary Figure 7. VOR learning induced with visual-vestibular pairing

Motor learning induced by pairing the same vestibular stimulus used in Figures 1, 2, and Supplementary Figure 3 with a moving visual stimulus. If the visual stimulus moved in the opposite direction from the head, the gain of the VOR increased during the 30-min training period (*upward triangles*; $36 \pm 10\%$, $t(11) = 3.528$, $P = 0.005$, one-sample t-test; $n = 12$). If the visual stimulus moved in the same direction as the head, the gain of the VOR decreased (*downward triangles*; $-45 \pm 5\%$, $t(7) = 8.745$, $P < 0.0001$, one-sample t-test; $n = 8$). The VOR was tested by briefly interrupting the pairing to measure the eye movement response to the vestibular stimulus in the absence of the visual stimulus. For comparison, habituation induced by the vestibular stimulus alone is indicated by the dotted trace. These experiments were performed on the same cohort of mice that underwent training with unilateral optogenetic Purkinje cell stimulation (Supplementary Figure 3). Mean \pm s.e.m.